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Viewpoint

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Detection of Thrombin with an Aptamer-Based Macromolecule Biosensor Using Bacterial Ghost System

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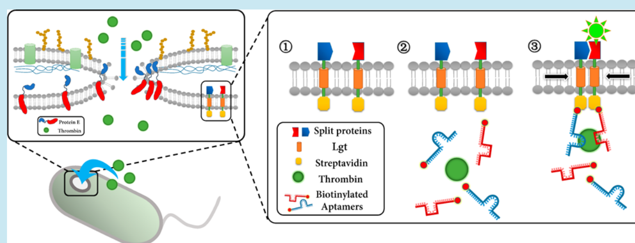
Supporting Information

ABSTRACT: A rapid on-site detection of exogenous proteins without the need for equipped laboratories or skilled personnel would benefit many areas. We built a rapid protein detection platform based on aptamer-induced inner-membrane scaffolds dimerization by virtue of bacterial ghost system. When the detection platform was coincubated with two kinds of aptamers targeting two different sites of thrombin, green fluorescence or β-lactamase activity were yielded with two different designs. The latter was detected by commercially available testing strips.



Single-stranded DNA or RNA can obtain high affinity toward a wide range of molecules with a combinational selection method called systemic evolution of ligands by exponential enrichment (SELEX). These binding nucleic acids are known as aptamers.[1] Specific detection of macromolecules such as proteins is restricted to the usage of antibodies. Although there have been reports that aptamers can be employed to detect proteins, elaborative redesigning and reselection of existing aptamers as well as sophisticated equipment are usually required.[2]

Bacterial ghosts are perforated envelopes of Gram-negative bacteria produced by expression of the cloned lysis gene *E* from bacteriophage ϕX174. Because the encoded product protein E will insert into the inner- and outer-membrane and fuse these two layers to produce orifices, bacterial ghosts are devoid of cytoplasmic contents but the periplasmic space is intact and the cellular morphology is preserved. As the outer-membrane possesses antigenic properties and the orifices are big enough to allow macromolecules to freely diffuse in and out, bacterial ghosts have been utilized for vaccine and drug delivery vehicles,[3] but the application as a biosensor platform has not been reported to our knowledge.

Our interest in developing a new approach for exogenous protein rapid and portable detection led to a synthetic biology strategy. We invented a sensor that is based on aptamer-induced inner-membrane scaffolds dimerization using bacterial ghost system.

RESULTS AND DISCUSSION

Construction of Bacterial Ghost Detection System. We first cloned the lysis gene *E* from bacteriophage ϕX174 DNA and cut it into a pBAD promoter regulated high expression vector to form part BBa_K1054001. The plasmid was then transformed into *E. coli* BL21 (DE3) cells, and growth curves were recorded under different concentrations of L-arabinose. An L-arabinose concentration of 0.4% was sufficient to induce cell lysis within 30 min (Supporting Information Figure S1). We also characterized the morphology of bacterial ghosts using both scanning electron microscope and transmission electron microscope. As previously observed, orifices with diameter around 100 nm were visible on the cell surface and the bacillus morphology of *E. coli* was preserved (Supporting Information Figure S1).

In our experiments, we tried to detect thrombin as a proof-of-concept attempt for our biosensor. We chose two well characterized aptamers, a 15-mer DNA aptamer recognizing thrombin fibrinogen binding exosite and a 29-mer DNA aptamer recognizing thrombin heparin binding exosite, and then connect them with 5′ poly(T) linkers to achieve optimal spatial position.[4] We also modified 5′ end of each aptamer with a biotin tag for the later on purpose of linking them to the inner-membrane scaffolds.

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The detection mechanism was based on the idea of aptamer-induced *E. coli* inner-membrane protein scaffolds dimerization. The inner-membrane scaffolds were fusion proteins composed of four domains from N-terminal to C-terminal of SRP-dependent exported protein DsbA (ssDsbA), split proteins, phosphatidylglycerol::prolipoprotein diacylglyceryl transferase (Lgt), and streptavidin. Lgt is an abundant *E. coli* endogenous trans-inner-membrane protein. ssDsbA can target the fusion protein to the inner-membrane through signal recognition particle (SRP) pathway and help to locate split proteins in the periplasm and streptavidin in the cytoplasm.[5] Because both 15-mer and 29-mer aptamers were biotinylated, they can attach to inner-membrane scaffold through biotin−avidin interaction. We conducted bacterial-ghosts based pull-down assay followed by western-blot to detect thrombin confirmed the construction of the system (Supporting Information Figure S2). In addition, as these two aptamers can recognize two sites of thrombin, they can drag two molecules of inner-membrane scaffolds into close proximity if thrombin is captured. When spatial distance is favored, split proteins in the periplasmic space will integrate into functional proteins whose activities are detectable.

Thrombin Detection Using Split EGFP. We expressed inner-membrane scaffolds with split EGFP fragments FA and FB fused to the periplasmic end.[6] The resulting parts, BBa_K1054017 and BBa_K1054018, were cleaved into coexpression vector pCDFDuet-1 and transformed into *E. coli* BL21 (DE3) cells to produce the corresponding fusion proteins. BBa_K1054001 was cotransformed to produce bacterial ghosts. Then, we tested the function of our membrane scaffolds by adding 29-mer and 15-mer aptamers to the bacterial ghost solution with 0.2 nM thrombin. The green fluorescence under confocal microscope (Figure 1c) was significantly brighter than the control groups to which either only aptamers (Figure 1a) or thrombin (Figure 1b) was added (Figure 1d, both $q < 0.01$, t test). Also, weak fluorescence was generated when single aptamer (19-mer or 25-mer) and thrombin were incubated with bacterial ghost (data not shown). All these results indicated a mechanism of thrombin-induced inner-membrane scaffolds dimerization.

Thrombin Detection Using β-Lactamase Testing Strips. Next, we split β-lactamase into to fragments BLF1 and BLF2 and fused them to the periplasmic end.[7] Likewise, the resulting parts, BBa_K1054019 and BBa_K1054020, were cleaved into coexpression vector pCDFDuet-1 and cotransformed with BBa_k1054001. Because the activity of β-lactamase can be detected with commercially available testing strips, this construction allowed rapid on-site strip detection of thrombin. When the concentration of thrombin in the solution was adjusted to 0.2 nM, a visible T-line indicating the existence of β-lactamase was visible (Figure 1e, II).

Discussion. In this study, we have shown an aptamer-based thrombin biosensor on the basis of bacterial ghost system with sensitivity of μg level comparable with Coomassie blue staining. The existence of thrombin can be easily illustrated via fluorescence or testing strips. This proof-of-concept experiment taking advantage of testing strips suggests a novel approach for macromolecule rapid on-site detection. Future efforts are focused on improving the sensitivity by increasing inner-membrane scaffolds expression level and extending detection targets to other macromolecules.

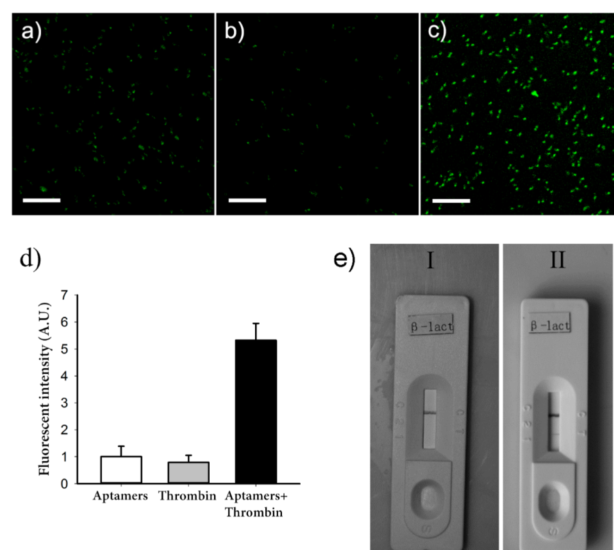

Figure 1. Detection of thrombin with the aptamer-based biosensor. Confocal microscope images of bacterial ghosts expressing inner-membrane scaffolds when only 29-mer and 15-mer aptamers were added (a) or only thrombin with final concentration of 0.2 nM was added (b) or both aptamers and thrombin were added (c). White markers in the bottom left corner of the images measures 10 μm. (d) The green fluorescence intensity was significantly stronger than the control groups where only either aptamers or thrombin was added (both $p < 0.01$, $n = 3$, t test. AU, arbitrary unit). The data shown are mean ± SD (e) β-lactamase testing strips. I shows a negative control with a visible C-line. When bacterial ghosts were coincubated with aptamers and thrombin, a T-line indicating β-lactamase activity was visible.

METHODS

Lysis Gene *E* and Inner-Membrane Scaffolds Gene Cloning. The lysis gene *E* was cloned from ΦX174 RF I DNA (TaKaRa) and put under control of pBAD promoter. The transmembrane protein phosphatidylglycerol::prolipoprotein diacylglyceryl transferase (Lgt) was cloned from *E. coli* BL21 (DE3) genome. Other genes were cloned from 2013 iGEM distribution kit. The construct of RBS-ssDsbA-split protein-lgt-streptavidin fusion protein was conducted using the circular circular polymerase extension cloning (CPEC) method by introducing overlapping sequence to each part. For the expression of fusion proteins, FFA and FFB were cloned into coexpression vector pCDFDuet-1 (Novagen) as well as FBLF1 and FBLF2.

Protein Expression and Aptamer Experiments. L-Arabinose (Aladdin) was used to induce the expression of protein E. Cells were then centrifuged and collected. For aptamer experiments, 10 μL 100 μM 29-mer aptamer, 10 μL 100 μM 15-mer aptamer, 10 μL bacterial ghost, and 10 μL 2 nM were added to a 60 μL PBS solution. The solution was then incubated at 37 °C for 30 min and prepared for subsequent experiments.

β-Lactamase Testing Strip. β-Lactamase testing strips (IVD Biotech) were conducted according to product instructions. Briefly, 10 drops of coincubated bacterial ghost solution were added to reaction solution provided by the product and incubated at 37 °C for 10 min. Then a drop of solution was moved to the strip's testing zone. Pictures were taken 10 min later.

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ASSOCIATED CONTENT

Supporting Information

Detailed experimental procedures for plasmid construction, bacterial ghost pull-down assay, protein expression protocols, and microscope cell imaging and testing strip protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

◆K.D., Y. C., L.Z., and Z.L. contributed equally. J.W. performed the molecular and microbiological work, designed the study, and wrote the manuscript. K.D., Y.C., and L.Z., were involved in experimental works and writing the manuscript. Z.L., W.G., and X.Y., performed preliminary experiments. A.X., X.L., J.H., C.X., and Y.C., participated in experimental planning and project background researching. M.C. supervised the study.

Notes

The authors declare no competing financial interest.

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